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Increased contractility of cardiomyocytes from copper-deficient rats is associated with upregulation of cardiac IGF-I receptor

Feng Dong,[1,*] Lucy B. Esberg,[2,*] Zamzam K. Roughead,[3] Jun Ren,[1,2] and Jack T. Saari[3]

[1]*Division of Pharmaceutical Sciences and Center for Cardiovascular Research and Alternative Medicine, University of Wyoming, Laramie, Wyoming;* [2]*Department of Pharmacology, Physiology and Therapeutics, School of Medicine, University of North Dakota; and* [3]*United States Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Center, Grand Forks, North Dakota*

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Dong, Feng, Lucy B. Esberg, Zamzam K. Roughead, Jun Ren, and Jack T. Saari. Increased contractility of cardiomyocytes from copper-deficient rats is associated with upregulation of cardiac IGF-I receptor. *Am J Physiol Heart Circ Physiol* 289: H78–H84, 2005; doi:10.1152/ajpheart.01093.2004.—Hearts from severely Cu-deficient rats show a variety of pathological defects, including hypertrophy and, in intact hearts, depression of contractile function. Paradoxically, isolated cardiomyocytes from these rats exhibit enhanced contractile properties. Because hypertrophy and enhanced contractility observed with other pathologies are associated with elevation of insulin-like growth factor-I (IGF)-I, this mechanism was examined for the case of dietary Cu deficiency. Male, weanling Sprague-Dawley rats were provided diets that were deficient (~0.5 mg Cu/kg diet) or adequate (~6 mg Cu/kg diet) in Cu for 5 wk. IGF-I was measured in serum and hearts by an ELISA method, cardiac IGF-I and IGF-II receptors and IGFBP-3 were measured by Western blotting analysis, and mRNAs for cardiac IGF-I and IGF-II were measured by RT-PCR. Contractility of isolated cardiomyocytes was assessed by a video-based edge-detection system. Cu deficiency depressed serum and heart IGF-I and heart IGFBP-3 protein levels and increased cardiac IGF-I receptor protein. Cardiac IGF-II protein and mRNA for cardiac IGF-I and IGF-II were unaffected by Cu deficiency. A Cu deficiency-induced increase in cardiomyocyte contractility, as indicated by increases in maximal velocities of shortening ($-dL/dt$) and relengthening ($+dL/dt$) and decrease in time to peak shortening (TPS), was confirmed. These changes were largely inhibited by use of H-1356, an IGF-I receptor blocker. We conclude that enhanced sensitivity to IGF-I, as indicated by an increase in IGF-I receptor protein, accounts for the increased contractility of Cu-deficient cardiomyocytes and may presage cardiac failure.

insulin-like growth factor-I

A VARIETY OF CARDIAC morphological and functional defects are observed in dietary Cu deficiency. Morphological defects include an increased heart size, connective tissue abnormalities, mitochondrial structural defects, and enlargement (30, 35). Observed functional defects include arrhythmia (23) and depression of contractile function in isolated hearts (2, 34) and, with one exception (29), in situ (11, 12, 17). Furthermore, findings in Cu-deficient hearts of the reexpression of fetal genes (19), enhanced apoptosis (20), and relative inability to respond to a β-adrenergic stimulus (11, 12) are consistent with imminent heart failure.

Paradoxically, contractility of isolated cardiomyocytes is increased in Cu-deficient rats (42). This implies compensation at a cellular level to account for the deficit in global heart function. It is possible that impaired function as measured in the whole heart may be a consequence of weakened connective tissue, which is known to occur in Cu deficiency (41) and which would not be manifest in an isolated cell. Whereas the delineation of the function of the whole heart in dietary Cu deficiency is the ultimate goal of our research, the goal of the present project was to determine the mechanism of enhanced contraction of isolated cardiac myocytes.

We were directed to a possible mechanism for increased cardiomyocyte contractility by the presence of cardiac hypertrophy in Cu deficiency. Because hypertrophy in other pathological conditions has been associated with an elevation of insulin-like growth factor-I (IGF-I) (7, 10, 26, 39), we hypothesized that cardiac IGF-I would be elevated in Cu-deficient hearts. IGF-I has been shown to have a positive inotropic effect on hearts (5), in particular in failing human hearts (40), therefore, an elevation of its concentration in Cu-deficient hearts would be consistent with the enhanced contractility observed in isolated heart cells. To examine this possibility, we measured relative amounts of IGF-I and its message, IGF-I receptor, IGF binding protein 3, as well as cardiomyocyte contractile responses in the presence and absence of IGF-I receptor blocker H-1356, in hearts of Cu-adequate and Cu-deficient rats.

METHODS

Animals and diets. Experiments were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* (31) and approved by the Animal Care Committee of the Grand Forks Human Nutrition Research Center.

Male, weanling Sprague-Dawley rats (Sasco, Lincoln, NE) were given access to either Cu-deficient or Cu-adequate diets. Three separate experiments were performed: *1*) IGF-I protein in serum and heart were measured; *2*) hearts were assayed for IGF-I and IGF-II receptor proteins, IGFBP-3 protein and mRNA of IGF-I and IGF-II; and *3*) cardiomyocyte contractile function was measured. The number of rats for each experiment is indicated in Table 1. The similarity of conditions between experiments is demonstrated by the similarity of Cu status indexes that were common to different groups of rats (Table 1).

Diets were composed of 940.0 g of Cu-free, iron(Fe)-free basal diet (catalog no. TD 84469, Teklad Test Diets, Madison, WI); 50.0 g of

* F. Dong and L. B. Esberg contributed equally to this work.

Address for reprint requests and other correspondence: J. T. Saari, USDA, ARS, Grand Forks Human Nutrition Research Center, Grand Forks, ND 58202-9034 (jsaari@gfhnrc.ars.usda.gov).

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Table 1. *Copper status indexes in Cu-adequate and Cu-deficient rats*

Dietary group	Serum and Heart IGF-I		IGF-I and IGF-II Receptors, IGFBP-3 and IGF-I, and IGF-II mRNA		Contractile Function of Isolated Heart Cells	
	CuA	CuD	CuA	CuD	CuA	CuD
n	10	10	8	8	3	3
Body weight, g	346±16	289±7	326±9	286±6*		
Liver Cu, μg/g dry wt	10.7±0.5	0.9±0.1*	11.8±0.2	1.0±0.1*	11.3±0.1	1.3±0.3†
Heart Cu, μg/g dry wt			23.0±0.4	6.6±0.5*		
Kidney Cu, μg/g dry wt	28.4±1.8	9.7±0.2*	30.2±1.4	10.8±0.3*	28.7±2.5	10.3±0.8*
Liver Fe, μg/g dry wt	217±10	289±21*	316±25	498±42*	272±25	428±13*
Hematocrit	0.44±0.01	0.25±0.02*	0.44±0.01	0.28±0.02*		
Heart weight, g	1.04±0.02	1.33±0.06*	0.99±0.03	1.36±0.08*		
Heart weight, mg/g body wt	3.06±0.14	4.63±0.26*	3.08±0.07	4.75±0.31*		

Values are means ± SE. CuA, Cu-adequate rat; CuD, Cu-deficient rat; IGF-I, insulin-like growth factor-I. *t-Test indicates significant difference ($P < 0.05$) from CuA animals.

safflower oil; and 10.0 g of Cu-Fe mineral mix per kilogram of diet. The basal diet was a casein (200 g/kg)-, sucrose (386 g/kg)-, cornstarch (295 g/kg)-based diet containing all known essential vitamins and minerals except for Cu and Fe (18). The mineral mix contained cornstarch and Fe with or without Cu and provided 0.22 g of ferric citrate (16% Fe) and either 0 or 24 mg of added $CuSO_4$ 5 H_2O/kg of diet. These formulations were intended to provide a severely Cu-deficient diet containing only Cu present in the basal diet and a Cu-adequate diet containing 6 mg Cu/kg of diet. Triplicate analysis (see below) of each of six diets (3 Cu-adequate and 3 Cu-deficient) used in the following experiments indicated that Cu-adequate diets ranged from 5.7 to 7.9 mg Cu/kg diet and Cu-deficient diets ranged from 0.2 to 0.3 mg Cu/kg diet.

Analysis of dietary Cu was performed by dry ashing of the diet sample (15), dissolution in aqua regia, and measurement by atomic absorption spectroscopy (model 503, PerkinElmer, Norwalk, CT). The assay method was validated by simultaneous assays of a wheat flour reference standard (National Institute of Standards and Technology, Gaithersburg, MD) and a dietary reference standard (HNRC-1A) that was developed by the Grand Forks Human Nutrition Research Center.

After the rats consumed their respective diets for 5 wk, each rat was anesthetized with an intraperitoneal injection of thiobutabarbital sodium (Inactin, 100 mg/kg body wt; Research Biochemicals International, Natick, MA).

Blood was withdrawn from the inferior vena cava into EDTA-treated test tubes, and the hematocrit was determined with a cell counter (model 3500CS, Cell-Dyn, Abbott Diagnostics, Santa Clara, CA).

Liver Cu and Fe, kidney Cu, and heart Cu (when sufficient tissue was available) were used as indexes of Cu status. Organ mineral concentration was determined by lyophilizing and digesting organ samples with nitric acid and hydrogen peroxide (32) and measuring Cu concentration by inductively coupled argon plasma emission spectroscopy (Optima 3100XL, PerkinElmer, Shelton, CT).

Measurement of serum and heart IGF-I. After removal of binding proteins with acid treatment, serum IGF-I was determined with an enzyme-immunosorbant assay kit (Diagnostic Systems Laboratory, Webster, TX). The inter- and intraassay variabilities for serum IGF-I were 4.9–11.9% and 5.3–9.1%, respectively.

Excised hearts were immediately frozen in liquid nitrogen and stored at −20°C until lyophilization. Concentrations of IGF-I were determined by using a modification of the procedure described previously (6). Briefly, the lyophilized organs were pulverized, and duplicate aliquots were extracted with 1 M acetic acid by end-to-end rotation for 4 h at 4°C. The extraction mixtures were centrifuged. After pretreatment to remove IGF-I binding proteins, the IGF-I concentration of the supernatant was determined by using a competitive binding enzyme immunoassay kit (Diagnostic Systems Laboratories, Webster, TX). The recovery of an internal standard of IGF-I added at the beginning of the extraction procedure was ~99% for both tissues.

Western blot analysis of heart IGF-I and IGF-II receptors and IGFBP-3. For Western blot analyses, tissues from rat ventricles were homogenized and lysed in RIPA lysis buffer: 20 mM Tris (pH 7.4), 1 mM EDTA, 1 mM EGTA, 150 mM NaCl, 1% Triton, 0.1% SDS, and protease inhibitor cocktail (Sigma P-8340, 1:100 dilution). Lysates were sonicated and clarified by being centrifuged at 13,000 *g* for 25 min at 4°C, and protein concentrations were determined by using the Bio-Rad protein assay reagent (Bio-Rad Laboratories, Richmond, CA). Protein samples were then mixed 1:2 with Laemmli sample buffer with 5% 2-mercaptoethanol and heated at 95°C for 5 min. SDS-PAGE was performed on a 7% (for IGF-I R and IGF-II R) and 15% (for IGFBP-3) acrylamide slab gels. SeeBlue Plus2 PreStained markers (Invitrogen, Carlsbad, CA) were used as standards. Electrophoretic transfer of proteins to nitrocellulose membranes (0.2 μm pore size, Bio-Rad Laboratories, Hercules, CA) was accomplished in a transfer buffer consisting of 25 mM Tris·HCl, 192 mM glycine, and 20% methanol for 60 min at 288 mA. Membranes were blocked for 60 min at room temperature in 20 mM Tris (pH 7.6), 137 mM sodium chloride, and 0.1% Tween-20 (TBST) with 5% nonfat dried milk. Membranes were incubated overnight with primary antibody at 4°C. Primary antibodies anti-IGF-IR, anti-IGF-IIR antibody (Cell Signaling, Beverly, MA), and anti-IGFBP-3 (Upstate, Lake placid, NY) were used at a dilution of 1:1,000. After incubation with a primary antibody, blots were incubated with either anti-mouse or anti-rabbit IgG horseradish peroxidase-linked antibodies at a dilution of 1:5,000 for 120 min at room temperature. After three washes in TBST, immunoreactive bands were detected by using the SuperSignal West Dura Extended Duration Substrate (Pierce, Milwaukee, WI). The intensity of bands was measured with a scanning densitometer (model GS-800; Bio-Rad) coupled with Bio-Rad PC analysis software.

mRNA measurement of IGF-I and IGF-II. To quantify IGF-I and IGF-II mRNA levels, total RNA was extracted from ventricles of the rat by using the guanidine thiocyanate method and was quantified spectrophotometrically at 260 nm (260/280 nm ratio ~1.9). Total RNA was treated with DNAse to prevent possible PCR amplification of chromosomal DNA contaminant. RT-PCR analysis was performed as follows: total RNA (4 μg) was reverse transcribed at 42°C for 50 min using 1 μl oligo(dt)12–18 (500 μg/ml) and 1 μl (200 units) super II reverse transcriptase (RT) (Invitrogen, Carlsbad, CA) in 20 μl reaction mixture containing 2 μl of 0.1 M dithiothreitol, 4 μl of 5× RT buffer, and 1 μl of 10 mM of each dNTP. The RT reaction was terminated by heating at 70°C for 15 min. One microliter of cDNA was amplified in 25 μl of a reaction mixture containing: 2.5 μl 10× PCR buffer, 0.75 μl 50 mM $MgCl_2$, 0.5 μl of 10 mM of each dNTP, 1 unit of platinum Taq DNA polymerase (Invitrogen, Carlsbad, CA), and 0.5 μl of the respective oligonucleotide primer pair (25 pM/μl).

The primers used were 5′-GCT CTT CAG TTC GTG TGT GG-3′ (forward) and 5′-TTG GGC ATG TCA GTG TGG-3′ (reverse) for rat IGF-I (accession numbers M15481, 221 bp), 5′-CGT GGA AGA GTG CTG CTT CC-3′ (forward) and 5′-GAC ATC TCC GAA GAG GCT CC-3′ (reverse) for IGF-II (accession numbers M29880, 329 bp), 5′-CAT CCT GAC CCT CAA GTA CCC-3′ (forward) and 5′-GTG GTG GTG AAG CTG TAG CC-3′ (reverse) for β-actin (accession numbers U39357, 420 bp) and were synthesized by IDT Integrated DNA Technologies (Coralville, IA). PCR for IGF-I and IGF-II consisted of initial denaturing at 94°C for 2 min; then at 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s; followed by 25 cycles with a final extension at 72°C for 7 min. PCR was performed in a P×2 PCR System (Thermo Hybaid). PCR products were separated by electrophoresis on 1.5% agarose gels and stained with ethidium bromide. The intensity of bands was measured with a scanning densitometer (model GS-800; Bio-Rad) coupled with Bio-Rad PC analysis software. The relative levels of PCR products were normalized by comparing with mRNA for β-actin.

Isolation of ventricular myocytes. Single ventricular myocytes were isolated from Cu-adequate or Cu-deficient rat hearts as described previously (36). One animal was killed each day, and the isolations were alternated between the control and experimental groups. In brief, hearts were rapidly removed and perfused (at 37°C) with oxygenated (5% CO_2-95% O_2) perfusion buffer that contained (in mM) 118 NaCl, 4.7 KCl, 1.25 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 25 $NaHCO_3$, 10 HEPES, and 11.1 glucose (pH 7.4). Hearts were subsequently perfused with a nominally Ca^{2+}-free perfusion buffer for 2–3 min (until spontaneous contractions ceased) and then were perfused for 20 min with Ca^{2+}-free perfusion buffer containing 223 U/ml of collagenase (Worthington Biochemical; Freehold, NJ) and 0.1 mg/ml of hyaluronidase (Sigma Chemical; St. Louis, MO). After perfusion, the left ventricle was removed, minced, and incubated with fresh enzyme solution (Ca^{2+}-free perfusion buffer containing 223 U/ml of collagenase) for 3–5 min. The cells were further digested with 0.02 mg/ml of trypsin (Sigma) before being filtered through a 300-μm nylon mesh and collected by centrifugation (60 *g* for 30 s). Myocytes were resuspended in a sterile filtered Ca^{2+}-free perfusion buffer containing (in mM) 131 NaCl, 4 KCl, 1 $MgCl_2$, 10 HEPES, and 10 glucose, supplemented with 2% BSA (pH 7.4, 37°C). Cells were initially washed with Ca^{2+}-free perfusion buffer to remove remnant enzyme, and extracellular Ca^{2+} was added incrementally to achieve a 1.25 mM concentration. Isolated myocytes were maintained at 37°C in a serum-free medium consisting of medium 199 (Sigma) with Earle's balanced salts containing 25 mM HEPES and $NaHCO_3$ supplemented with 2 mg/ml of BSA, 2 mM L-carnitine, 5 mM creatine, 5 mM taurine, 5 mM glucose, 0.1 μM insulin, 100 U/ml of penicillin, 100 mg/ml of streptomycin, and 100 mg/ml of gentamycin. Mechanical properties remained relatively stable in myocytes maintained for 12–24 h in the serum-free medium. Cells that had any obvious sarcolemmal blebs or spontaneous contractions were not used; only rod-shaped myocytes with distinctly clear edges were selected for recording of mechanical properties as previously described (37).

Cardiomyocyte shortening and relengthening. Mechanical properties of ventricular myocytes were assessed by using a video-based edge-detection system (IonOptix; Milton MA) as previously described (36). In brief, coverslips with cells attached were placed in a chamber mounted on the stage of an inverted microscope (Olympus X-70) and superfused (~2 ml/min at 25°C) with a buffer containing (in mM): 131 NaCl, 4 KCl, 1 $MgCl_2$, 10 glucose, and 10 HEPES (pH 7.4). The cells were field stimulated at a frequency of 0.5 Hz for 3 ms in duration using a pair of platinum wires placed on opposite sides of the chamber connected to a stimulator (FHC; Brunswick, ME). The polarity of the stimulating electrodes was reversed periodically to avoid potential buildup of electrolysis by-products. The myocyte being studied was displayed on a computer monitor using an IonOptix MyoCam camera, which rapidly scans the image area every 8.3 ms such that the amplitude and velocity of shortening or relengthening are recorded with good fidelity. Changes in cell length (CL) during shortening and relengthening were captured and converted into an analog voltage signal. Cell shortening and relengthening were assessed using indexes of peak shortening (PS), time to PS (TPS), time to 90% relengthening (TR_{90}), and maximal velocities of shortening ($-dL/dt$) and relengthening ($+dL/dt$) (37). Myocytes were allowed to contract at a stimulation frequency of 0.5 Hz over 10 min to ensure steady state before the mechanical function was recorded. In some experiments, myocytes were preincubated with the IGF-I receptor antagonist IGF-I Analog H-1356 (20 μg/ml, Bachem Bioscience, King of Prussia, PA) for 4 h (36) before mechanical recording. The H-1356 compound blocks the IGF-I receptor by specifically inhibiting its autophosphorylation by IGF-I (33).

Statistics. The Student's *t*-test was used to compare variables of Cu-adequate and Cu-deficient animals. To examine effects of Cu status and IGF-I inhibition on functional variables of isolated cardiomyocytes, data were analyzed by using a mixed model analysis of variance (Proc Mixed in SAS) with rat as a random effect and diet and treatment as fixed effects. When the interaction between diet and treatment was statistically significant ($P < 0.05$), Tukey's contrasts were used to compare the group means. Differences were regarded as significant at $P < 0.05$.

RESULTS

Copper status. Table 1 presents the Cu status indexes for three groups of rats used for measurement of *1*) serum and heart IGF-I, *2*) heart IGF-I and IGF-II receptors, IGFBP-3 and mRNA for IGF-I and IGF-II, and *3*) contractile properties of isolated heart cells. Changes previously shown to occur with severe dietary Cu deficiency, including depressed body weight gain, organ Cu and hematocrit, and elevated liver Fe and heart size (22), verify that all animals used were Cu deficient.

IGF-I in serum and heart. Cu deficiency reduced serum IGF-I protein concentration by 27% (Fig. 1*A*) and heart IGF-I by 34% (Fig. 1*B*).

Cardiac IGF-I and IGF-II receptors and IGFBP-3. Western blot analysis (Fig. 2*A*) indicated that the receptor for IGF-I was elevated (41%), the receptor for IGF-II was unchanged, and IGFBP-3 was reduced (17%) in hearts of Cu-deficient rats (summarized in Fig. 2, *B–D*).

Cardiac mRNA for IGF-I and IGF-II. RT-PCR analysis (Fig. 3*A*) indicated that the cardiac mRNA for neither IGF-I nor IGF-II was affected by dietary Cu deficiency (summarized in Fig. 3, *B–C*).

Effect of IGF-I receptor blockade on cardiomyocyte contraction. No recognizable differences in myocyte yield, field stimulation threshold, spontaneous twitch, or viability were observed among the four treatment groups. Twenty cells were randomly selected for mechanical studies for each of three animals per group. Their functional characteristics are described in Table 2. Resting CL examined by ANOVA was not affected by diet. Amount of cell shortening in response to field stimulation (ΔCL) showed no effect of diet or IGF-I receptor blockade either in absolute value or change as a percentage of resting CL. Contractility, as estimated by maximum velocity of shortening and relengthening ($\pm dL/dt$), was enhanced by dietary Cu deficiency and reduced to control levels by H-1356 (20 μg/ml) treatment. This effect was mirrored by the TPS, which was shortened by Cu deficiency and appeared to be partially corrected by H-1356 treatment. TR_{90} showed no effect of diet and a significant effect of H-1356 (ANOVA)

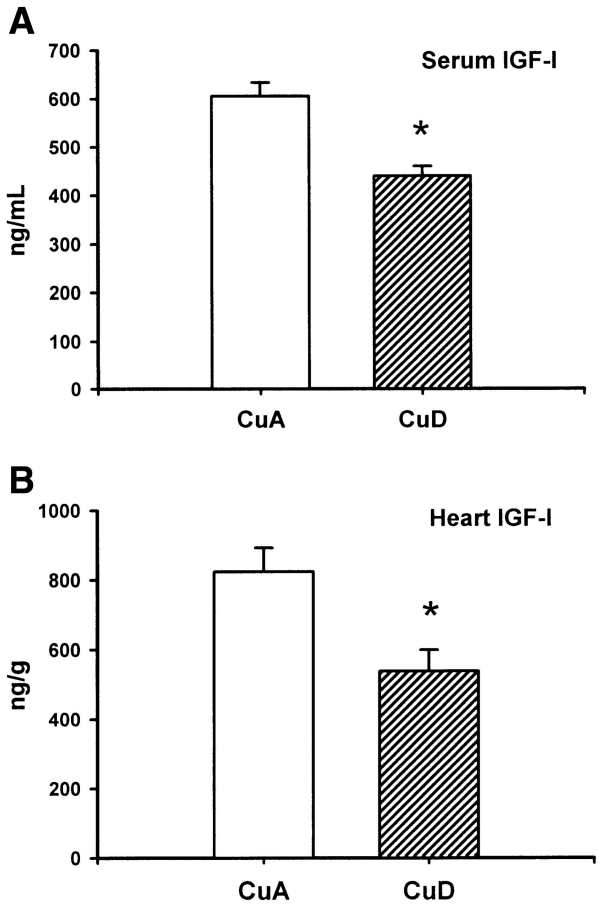

Fig. 1. Insulin-like growth factor-I (IGF-I) protein in serum (*A*) and heart (*B*) as estimated by an ELISA method. CuA, Cu adequate; CuD, Cu deficient. IGF-I showed a 27% depression in serum and a 34% depression in hearts from CuD rats. Values are means ± SE; $n = 10$ per group. *$P < 0.05$.

primarily because of its elevation by H-1356 in Cu-adequate rats.

DISCUSSION

This study confirmed the enhancement of contractile function observed previously in cardiomyocytes of severely Cu-deficient rats (42). This was evidenced by increases in rates of myocyte shortening and relengthening and also by the reduced TPS (Table 2).

The increased myocyte contractility is contradictory to the finding that whole heart function, measured in isolation (2, 34) or in situ (11, 12), is depressed in severe Cu deficiency. Although paradoxical, this finding is not entirely unexpected. Work by others (4, 9) suggests that failing hearts can compensate for global failure by upregulating so-called survival pathways. The possible outcomes of this upregulation include, in addition to delayed cell death, enhanced cellular function and an accompanying cardiac hypertrophy (8, 13, 16, 21, 28). Thus the enhanced myocyte contractility and the hypertrophy of whole hearts (Table 1) support the possibility of induction of compensatory survival pathways in the cardiomyopathy of dietary Cu deficiency.

Survival pathways induced by cardiomyopathy may include those involving IGF-I axis and related proteins. Haq et al. (16) have shown that the PI3K/Akt pathway, which is induced by IGF-I, is upregulated in failing hearts, as is the IGF-I receptor. Furthermore, IGF-I protein and message have been shown to be elevated in hypertrophic obstructive cardiomyopathy (26, 27), in human cardiac hypertrophy caused by aortic stenosis

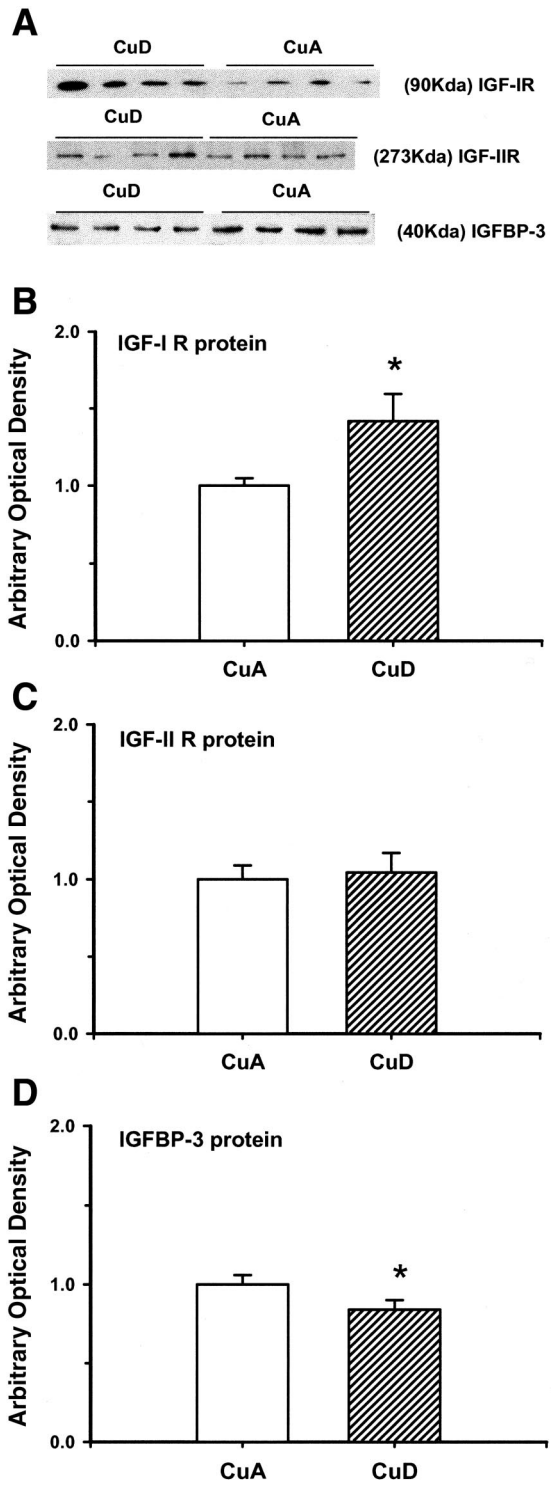

Fig. 2. Protein expression of cardiac IGF-I and IGF-II receptors and IGFBP-3 by Western blotting (*A*). Receptor for IGF-I was elevated by 41% (*B*), the receptor for IGF-II was unchanged (*C*) and IGFBP-3 was reduced by 17% (*D*) in hearts of Cu-D rats. Values are means ± SE; $n = 8$ per group. *$P < 0.05$.

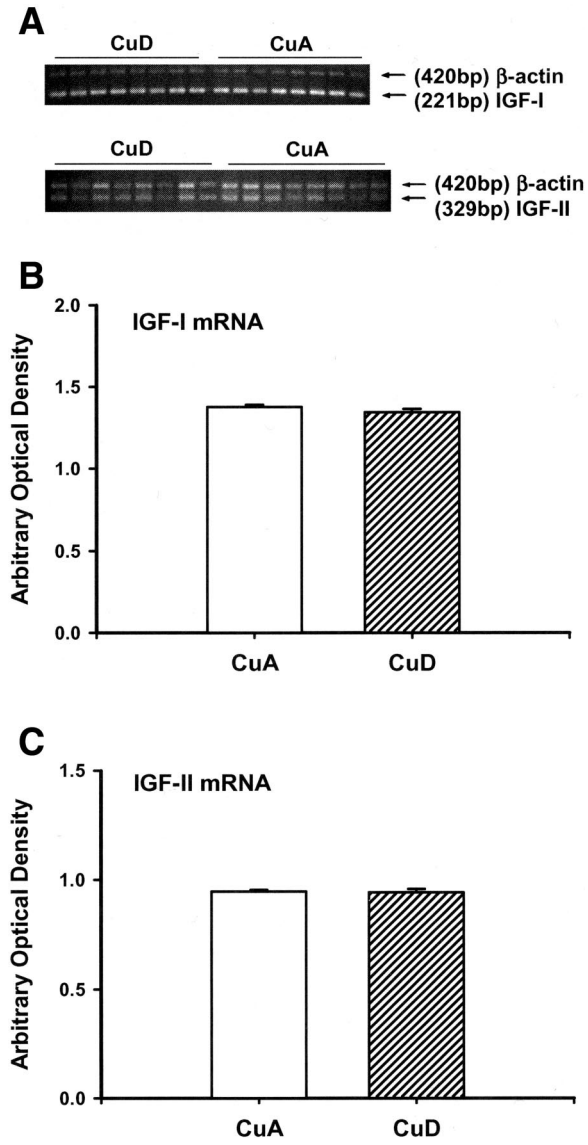

Fig. 3. Cardiac mRNA levels of IGF-I and IGF-II by RT-PCR (*A*). Cardiac message for neither IGF-I (*B*) nor IGF-II (*C*) was affected by dietary Cu deficiency. Values are means ± SE; $n = 8$ per group.

and aortic regurgitation (39), and in an animal model of heart failure induced by infarct (7). That IGF-I participates in the enhanced myocyte contractility, and perhaps in the hypertrophy, of Cu deficiency is indicated by the complete inhibition of the increases in maximum rates of shortening and relengthening and the partial inhibition of TPS by the IGF-I inhibitor H1356 (Table 2). That the PI3K/Akt pathway may be involved is supported by the knowledge that H-1356 can inhibit other processes known to be dependent on Akt phosphorylation. Specifically, H1356 has been shown to impair IGF-1-mediated inhibition of apoptosis (25), and induction of message for vascular endothelial growth factor (14), both of which, if operating in the heart, could be regarded as protective against cardiac failure.

In an effort to explain the enhanced myocyte contractility, we examined, by a variety of measures, how IGF-I metabolism was changed by Cu deficiency. Both plasma and cardiac concentrations of IGF-I were depressed (Fig. 1), although the message for cardiac IGF-1 was unchanged (Fig. 3*B*). Cardiac IGFBP-3, the primary carrier protein of IGF-I, was depressed by Cu deficiency (Fig. 2*D*). These findings suggest that the elevation of contractility was not caused by an elevated production of IGF-I. The IGF-I receptor concentration, however, was elevated (Fig. 2*B*), suggesting that the increased contractility was caused by an enhanced cardiac sensitivity to IGF-I, a possibility that warrants further examination.

A similar localized compensatory response to Cu deficiency that involves IGF-I has been reported for the bone (38). Under conditions similar to those of the present study, low Cu intake caused a significant reduction in the circulating levels of serum IGF-I (~30%) and in the breaking strength of long bones. But bone IGF-I was the highest in the animals fed the lowest Cu diets. This increase in bone IGF-I concentration, despite a systemic reduction of this peptide, suggests a localized compensatory response by the bone to its impaired function. Although different from findings of the present study in that IGF-I rather than IGF-I receptor is increased, the results for bone confirm the involvement of IGF-I in tissue compensation for functional defects caused by Cu deficiency.

Less is known about the role of IGF-II in cardiomyopathy. Although IGF-II has been shown to have hypertrophic properties (1) and can protect against impaired structure and function following infarct (3, 24), in at least one instance of cardiomyopathy and one of experimental hypertrophy, in both of which cardiac IGF-I message levels were increased, the message for IGF-II was unaffected. Such is the case in the

Table 2. *Effect of IGF-1 inhibition (H-1356) on characteristics of cardiomyocytes from Cu-adequate and Cu-deficient rats*

	Untreated		H-1356		Significance by ANOVA		
Dietary Group	CuA	CuD	CuA	CuD	Diet	H-1356	Diet × H-1356
Resting CL, μm	150±3	161±4	152±3	160±5	NS	NS	NS
ΔCL, μm	9.5±0.5	9.8±0.5	9.5±0.6	9.1±0.7	NS	NS	NS
ΔCL, %	6.4±0.4	6.3±0.4	6.3±0.4	5.8±0.4	NS	NS	NS
−d*L*/d*t*, μm/s	−104±6	−132±8	−107±7	−107±8	NS	NS	<0.05
+d*L*/d*t*, μm/s	90±6*	119±8†	89±7*	86±7*	NS	<0.02	<0.02
TPS, ms	138±4*	111±4†	136±4*	123±3*‡	<0.04	NS	<0.05
TR_{90}, ms	218±10	194±13	265±20	205±12	NS	<0.04	NS

Values are means ± SE. CL, cell length; ΔCL, change in cell length with field stimulation; ±d*L*/d*t*, maximal velocities of shortening (−) and relengthening (+); TPS, time to peak shortening; TR_{90}, time to 90% relengthening; NS, not significant. *†‡Different superscripts between values in a given row indicate a significant difference ($P < 0.05$).

present study where neither the message for cardiac IGF-II (Fig. 3*C*) nor the IGF-II receptor (Fig. 2*C*) was altered by dietary Cu deficiency.

In summary, we have confirmed the enhancement of contractility observed previously in cardiomyocytes of Cu-deficient rats and demonstrated its inhibition by treatment with an IGF-I receptor inhibitor. The findings that IGF-I production appears to be reduced and that the IGF-I receptor is increased suggest that the enhanced contractility is caused by an enhanced sensitivity to IGF-I. Based on literature evidence that survival pathways are induced in a variety of cardiomyopathies and that IGF-I can contribute to such pathways, we feel that further examination of this possibility is warranted for the cardiomyopathy of dietary Cu deficiency. Inasmuch as survival pathways are initiated when hearts tend toward failure, the findings support previous data (11, 12, 19) that severe Cu deficiency can contribute to heart failure. Whether such is true in marginal deficiency remains to be examined.

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